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TECHNICAL MANUSCRIPT 195

A NEW RACK FOR USE IN CELL CULTURE

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A NEW RACK FOR USE IN CELL CULTURE

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ABSTRACT

This describes a new rack with a variety of uses and applications in tissue culture and virology. Its invention was prompted by a need for a simple method of cloning cells. The principal feature of the rack is that it is designed to fit onto the stage of the inverted microscope.

A NEW RACK FOR USE IN CELL CULTURE

This describes a new rack with a variety of uses and applications in tissue culture and virology. Its invention was prompted by a need for a simple method of cloning cells. We started by using the tube method of cloning in a rack of the type offered by Microbiological Associates. In that method the tubes are incubated at an angle of 5 degrees to allow the cells to grow on one wall of the tube. The tube can then be removed from the rack, placed on a special observation track, and viewed in the microscope.

It occurred to us that the whole procedure might be simplified if the cells were grown on the floor of the tube rather than on the wall so that the cells could be examined directly in the inverted microscope without removing the tubes from the rack. This necessitated a flat-bottomed tube, i.e., a vial. Accordingly, we designed a rack (Figure 1) to fit onto the stage of the inverted microscope. The rack holds 40 shell vials (Kimble 60930, outside diameter 15 mm, height 45 mm). It is constructed of type 304 stainless steel, 22-gauge, and has holes 19/32 inch in diameter to position the vials. The holes in the floor of the rack are 9/16 inch in diameter, just small enough to support the vials without their falling through the rack and yet large enough to permit viewing the entire inside floor of the vial with the inverted microscope. The rows of vials are protected from airborne contamination by 3M Micropore surgical tape which is translucent. The rack is 11 9/16 inches long and 4 inches wide. The total height, including vials, is 1 13/16 inches. There is thus no space problem in the carbon dioxide incubator.

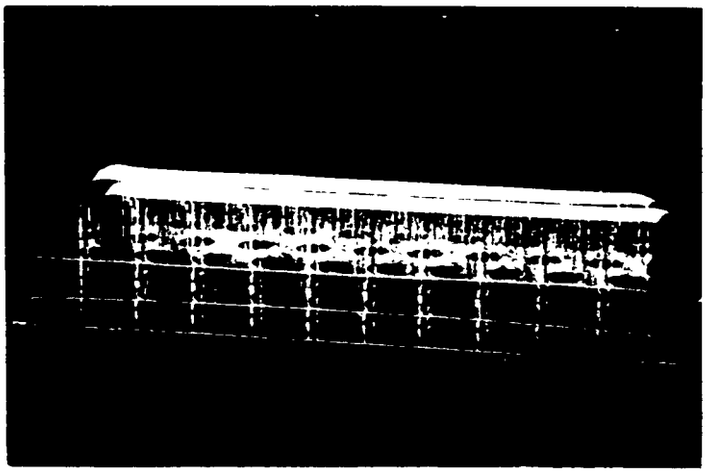

Figure 1. Rack, showing holes, vials, and tape.
(FD Neg C-7642)

Two experiments illustrate applications of the new rack. In the first experiment, a suspension of Syrian hamster 113 cells* was diluted to 4 cells per 10 ml. Two ml of this suspension was distributed to each of 40 vials and the rack was incubated at 37 C in a carbon dioxide incubator. After 7 days the whole rack was placed on the stage of the inverted microscope and each vial was examined for the presence of colonies (simply by moving the rack). The results are shown in Table 1. One vial was contaminated. The 14 vials containing one colony each were separated from the rest by cutting the tape and removing the vials from the rack. The colonies were refed and grown into cultures directly in the vials, thus avoiding any special techniques of isolation from other clones.

TABLE 1. CLONING OF HAMSTER 113 CELLS IN THE VIAL RACK

	Number of colonies[a]/ per vial			
	0	1	2	3
Number of vials found with 0, 1, 2 colonies, etc.	23	14	2	0
Number of vials expected[b]/	25	11	3	0

a. Cell input per vial 0.8.
b. Calculated according to the Poisson distribution.

This rack is also useful for other purposes, for example, the titration of viruses by production of cytopathic effect in monolayer cultures. In the second experiment 200,000 cells of strain 113 were transferred to each of 50 vials and the vials were incubated for two hours. A virus sample of known plaque-forming titer was serially diluted and the dilutions were inoculated into the vials in rows of 10 vials each. After 40 to 60 hours the rack was placed on the stage of the inverted microscope and the cultures were scored as showing cytopathic effect or not. The results of the experiment are shown in Table 2.

* Supplied by Dr. Vittorio Defendi, Wistor Institute, Philadelphia, Pa.

TABLE 2. ASSAY OF EASTERN EQUINE ENCEPHALITIS VIRUS BY PRODUCTION OF CYTOPATHIC EFFECT IN HAMSTER 113 CELLS

Virus dilution	Number of cultures showing cytopathic effect/normal
No virus	0/10
10^{-7}	10/0
10^{-8}	10/0
10^{-9}	3/7
10^{-10}	0/10

The vial rack has been in use in our laboratory for several months and has appreciably reduced the time required to perform a variety of tasks. It should be especially useful in genetic isolation of mutants, both cell and viral.